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Antibodies to meningococcal H.8 (Lip) antigen fail to show bactericidal activity

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Purified H.8 (Lip) antigen was coupled to tressyl-activated Sepharose 4B and used in affinity columns to purify anti-Lip antibodies from convalescent patient sera and from immune rabbit sera. Affinity-purified anti-Lip antibodies isolated from two convalescent patient sera contained 1000 and 1280 ELISA units of antibody and included antibodies of IgG, IgA, and IgM isotypes. An anti-Lip mouse monoclonal ascites (2-1-CA2) had 28 400 ELISA units of antibody. Bactericidal assays were performed using three different case strains of *Neisseria meningitidis* group B, namely 44/76, 8532, and 8047. Neither preparation of purified human anti-Lip antibodies had detectable bactericidal activity against strains 44/76 and 8532, but one of the two had a titer of 1:4 against strain 8047. Anti-Lip antibodies that were purified from immune rabbit serum and contained 1600 ELISA units of anti-Lip antibodies also failed to show detectable bactericidal activity. The rabbits were immunized with purified Lip antigen and showed specific antibody levels of 2000-2200 units by ELISA, but even the unfractionated sera had little or no bactericidal activity against the test strains. The high titer mouse monoclonal ascites had no bactericidal activity against the test strains. The poor bactericidal activity associated with monoclonal and polyclonal antibodies to the Lip antigen suggest that in spite of other attractive properties it may not be useful as a meningococcal vaccine.

Key words: anti-Lip, antibodies, bactericidal, *Neisseria*, Lip.

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L'antigène H.8 (Lip) purifié a été couplé à du Sepharose 4B activé au trésyl et utilisé en chromatographie d'affinité pour purifier des anticorps anti-Lip à partir de sérums de convalescents et de lapins immuns. Les anticorps anti-Lip purifiés à partir de deux sérums de convalescents avaient des titres de 1000 et 1280 unités ELISA et contenaient des IgG, des IgA et des IgM. Des liquides d'ascite (2-1-CA2) de souris riches en anticorps monoclonaux anti-Lip contenaient 28 400 unités d'anticorps en ELISA. Le pouvoir bactéricide a été mesuré sur trois souches cliniques de *Neisseria meningitidis* du groupe B, soit 44/76, 8532 et 8047. Aucune des préparations d'anticorps anti-Lip purifiés ne possédait d'activité bactéricide contre les souches 44/76 et 8532 mais une des préparations avait un titre de 1:4 contre la souche 8047. Aucune activité bactéricide n'a été détectée avec les anticorps anti-Lip de lapin purifiés malgré un titre de 1600 unité ELISA. Des lapins ont été immunisés avec l'antigène Lip purifié et ont produit des sérums dosant à 2000-2200 unités ELISA, mais même avec ces sérums non fractionnés, peu ou pas d'activité bactéricide n'a été observée contre les souches bactériennes testées. Les liquides d'ascite de souris riches en anticorps monoclonaux n'avaient pas non plus d'activité bactéricide contre ces souches. La faible activité bactéricide associée aux anticorps monoclonaux et polyclonaux dirigés contre l'antigène Lip laisse croire que, malgré certaines autres propriétés intéressantes, cet antigène est inutile comme vaccin contre la méningocoque.

Mots clé : anticorps, anti-Lip, bactéricidie, *Neisseria*, Lip.

[Traduit par la revue]

Introduction

The H.8 antigen was first discovered by Cannon et al. (1974) and has recently been renamed Lip (Hitchcock 1989), which is an abbreviation for lipoprotein. The Lip antigen is an unusual lipoprotein that is common to all pathogenic *Neisseria* species as well as some strains of *Neisseria lactamica* and *Neisseria cineria*, but it is generally not present on the other commensal *Neisseria* species (Cannon et al. 1984; Zollinger et al. 1985). It elicits a reasonably good antibody response in patients with meningococcal and gonococcal infections (Black et al. 1985). Using purified Lip as antigen in an ELISA, we found that a group of 10 patients with group B meningococcal disease had a geometric mean 15-fold rise in antibodies to Lip (Bhattacharjee et al. 1988a).

The question of surface exposure of Lip has been investigated by several methods, but the results are not definitive. It appears that the antigen may be exposed on some cells but not others (Hitchcock et al. 1985; Robinson et al. 1987). Strittmatter and Hitchcock (1986) have purified and characterized the gonococcal Lip and shown it to be an unusual lipoprotein, rich in alanine and proline and lacking aromatic amino acids and methionine.

We have purified and characterized meningococcal Lip using a different method (Bhattacharjee et al. 1988a) and found it to be similar to the gonococcal Lip. The three amino acids, alanine, proline, and glutamic acid, accounted for over 80% of the total amino acids present.

The Lip genes in both the gonococcus and the meningococcus have been cloned (Black and Cannon 1985; Gotschlich et al. 1986), and it has been found that two genes code for proteins that bind the anti-H.8 monoclonal antibodies. One gene codes for the Lip outer membrane protein and the other codes for a lipid-modified azurin designated Laz (Hitchcock 1989). The predicted amino acid sequence derived from the DNA sequence of the gonococcal *lip* gene reveals a structure composed of 13 to 14 repeats of the five amino acid consensus sequence Ala-Ala-Glu-Ala-Pro. Some of the repeats are not perfect, and the number of repeats appears to vary somewhat from strain to strain (Cannon 1989). The meningococcal *lip* gene has not yet been sequenced, but based on the amino acid composition of the purified meningococcal Lip (Bhattacharjee et al. 1988a), it is expected to be very similar.

The presence of the Lip antigen on all pathogenic *Neisseria* suggests that it might be involved in pathogenesis, and its stability and immunogenicity further suggest that it might

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function as an effective vaccine for meningococcal or gonococcal disease. To function as an effective vaccine an antigen must induce protective antibody. In the case of meningococcal disease, human immunity has been closely correlated with the presence of serum bactericidal antibodies (Goldschneider et al. 1969a, 1969b). It is therefore important to determine if antibodies (particularly human antibodies) to Lip are bactericidal and if they are protective in animal models. Hitchcock et al. (1987) showed that the H.8 monoclonal antibody McAb 10 was not bactericidal for strains of serum resistant gonococci, but had weak bactericidal activity against certain serum-sensitive gonococcal strains. Although other groups including our own have obtained several monoclonal antibodies with specificity for Lip, none have reported these antibodies to have bactericidal activity against meningococci. Although Woods et al. (1987) initially reported protection by anti-Lip monoclonal antibodies in a mouse model, they later found that the apparent protection was nonspecific and caused by contaminating endotoxin in the antibody preparation. We report here the affinity purification of anti-Lip antibodies from human convalescent sera, immune rabbit serum, and mouse ascites and our finding of the poor bactericidal activity of these antibodies against several meningococcal group B strains.

Materials and methods

Preparation of Lip affinity columns

Lip was purified by the procedure described previously (Bhattacharjee et al. 1988a). Its purity was estimated at greater than 95% based upon analysis by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS–PAGE) with silver staining by a sensitive method that reveals Lip as a distinctive yellow band, and upon the amino acid analysis of the purified antigen which agreed closely with the published amino acid composition predicted from the DNA sequence of the gonococcal *lip* gene (Cannon 1989). Purified Lip was linked to Sepharose 4B as follows: tressyl-activated Sepharose 4B (0.4 g; Pharmacia Fine Chemicals, Piscataway, NJ) was suspended into 1 mM HCl and washed on a sintered-glass funnel with 80 mL of 1 mM HCl over a period of 1 h. Purified Lip (1 mg) was taken into 2.0 mL of coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl, pH 8.3) and mixed with the washed tressyl-activated Sepharose 4B. The mixture was shaken for 2 h at room temperature and was then kept overnight at 5°C. The gel was washed once with coupling buffer. The excess active groups were blocked by treatment with 0.1 M Tris–HCl buffer pH 8.3 for 2 h at room temperature with shaking. The gel suspension was then washed on the sintered-glass filter with three alternate cycles of pH 4.2 buffer (0.1 M sodium-acetate, 0.5 M NaCl) and pH 8.1 buffer (0.1 M Tris, 0.5 M NaCl) and finally with PBS pH 7.5 (0.01 M sodium phosphate, 0.14 M NaCl, 0.02% sodiumazide). The gel was packed into a small column and stored at 5°C.

Isolation of anti-H.8 antibodies

The Lip affinity column was first tested for its capacity to retain anti-Lip antibodies. Mouse monoclonal ascites was diluted 10-fold in phosphate-buffered saline (PBS) and filtered through 0.45-µm Millipore membrane. This solution (0.5 mL) was loaded onto the washed H.8 affinity column (bed volume, 2 mL) and allowed to stand for 10 min at room temperature. The column was then washed with PBS until the absorbance of the eluate at 280 nm was less than 0.01 (6–8 mL). Anti-Lip antibodies were then eluted with 3.0 M potassium thiocyanate (KCNS) solution (pH 5.8) and six 1.0-mL fractions were collected. The column wash fractions and eluted fractions were assayed for the presence of anti-Lip antibodies by the ELISA procedure described below. Convalescent patient sera and immune rabbit sera were loaded onto the Lip affinity column without dilution. If anti-Lip antibodies were found in the column wash fractions (which happened after prolonged use of a column for about 6 months), the column was discarded and a fresh Lip affinity column was made as described above.

The column fractions containing anti-Lip antibodies were immediately desalted on a washed PD-10 column (Pharmacia Fine Chemicals, Piscataway, NJ) with PBS pH 7.4 to remove thiocyanate before testing for any activity.

ELISA

The ELISA was performed in 96-well flat-bottom polystyrene microtiter plates (Costar, Cambridge, MA), essentially by the method of Engvall and Perlmann (1972). The wells were coated with antigen in PBS, at a concentration of 10 µg/mL at 37°C for 3 h. Excess reactive sites were blocked with 0.5% casein and 0.5% BSA in PBS at 37°C for 1 h. The wells were washed with PBS containing 0.05% Tween 20 to avoid nonspecific adsorption. The antibodies used were mouse anti-Lip monoclonal antibodies (2-1-CA2), convalescent sera obtained from patients with *N. meningitidis* group B infection (kindly provided by Dr. Alfred Halstensen, Bergen, Norway), and immune rabbit sera obtained as described below. The antigen-coated plates were incubated with serial 2-fold dilutions of antibodies for 16 h at room temperature (25°C). Phosphatase-labeled goat anti-mouse, anti-human, and anti-rabbit secondary antibodies were obtained from Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD. Incubation with the second antibodies was done for 16 h at room temperature. The primary and secondary antibodies were diluted in PBS containing 0.5% BSA and 0.5% casein. P-Nitrophenyl phosphate disodium (Sigma Chemical Company, St. Louis, MO) at a concentration of 1 mg/mL in 1.0 M diethanolamine buffer pH 9.8 containing 1 mM MgCl₂ was used as substrate. Absorbances were read on a Dynatech plate reader (Dynatech Laboratories, Alexandria, Va) at 410 nm and absorbance readings greater than or equal to 0.40 were taken as positive. Antibody units were calculated as the mean of the product of the optical density and the reciprocal serum dilution at two different 2-fold dilutions in the range of 0.3 to 1.2.

Immunization of rabbits

New Zealand white rabbits (2–2.5 kg) were injected intramuscularly on both shoulders with purified Lip (30 µg mixed with Al(OH)₃ gel, at a gel to protein ratio of 30:1, w/w). Booster injections (containing 40 µg Lip in 0.15 M NaCl) were given i.v. 5 weeks later. A second booster injection was given the same way 4 weeks after the first. Rabbits were bled from the ear artery at 0, 5, 7, 9, and 13 weeks. These serum samples were tested for anti-Lip antibodies by the ELISA method.

Bacterial strains

Three strains of *N. meningitidis* group B and one of group C were used in this study. All were case strains and have been well characterized and extensively used in bactericidal assays in this laboratory. Strain 44/76(B:15:P1.16) was obtained from L. Oddvar Froholm, Oslo, Norway, and strains 8047(B:2b:P1.2), 8532(B:15:P1.3), and 60E(C:16:P1.1) are from our departmental culture collection. The information given in parentheses after the strain numbers indicates the serogroup:serotype:subtype of the strains in accordance with a recently proposed nomenclature for designating meningococcal antigens (Frasch et al. 1985).

Bactericidal assay

Bactericidal assays were performed essentially by the procedure described previously (Zollinger and Mandrell 1983). Briefly, bacteria were grown in a candle jar overnight at 37°C on GC agar with 1% defined supplement as described by Kellog et al. (1963) but with added cysteine and ferric nitrate (0.0017 and 0.001% (w/v), respectively, final concentration in the medium). Bacteria were inoculated into Mueller–Hinton broth to an OD at 650 nm of 0.1 and grown in a shaking water bath to an OD of 0.50 at 650 nm. The bacteria were pelleted at 5000 rpm for 10 min, and washed once with Gey's balanced salt solution (Microbiological Associates, Walkersville, MD) containing 0.1% gelatin. The bacteria were resuspended in the same buffer to an OD of 0.50. Serial 10-fold dilutions were made from this stock solution to obtain 1000 meningococci in 25 µL of solution. Tests were performed in 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA). The total volume in each well was 0.1 mL (25 µL each of buffer, serum, bacteria, and complement). Controls included (i) a

positive control containing serum with known bactericidal activity, (ii) a control for complement killing that contained active complement but buffer in place of serum, (iii) a negative control containing buffer in place of complement and serum, and (iv) controls containing heat-inactivated complement, bacteria, buffer, and serum (heat inactivated) or purified antibodies. After incubation at 37°C for 1 h viability was determined as colony-forming units by plating 20- μ L samples in triplicate from each well on GC agar with defined supplement. Plates were incubated in candle extinction chambers at 37°C overnight before counting colonies. It was found that storage of isolated anti-Lip antibodies in PBS at 5°C for 2 weeks caused denaturation. All tests were therefore performed on freshly isolated antibodies. Exogenous rabbit complement was used with the rabbit antibodies and human complement with the human antibodies.

Colony blot assay

Bacteria were grown on GC agar with defined supplement overnight at 37°C in a candle jar. Isolated colonies were suspended in Gey's buffered salt solution containing 0.2% gelatin to an OD of 1.07 at 650 nm. Five serial 10-fold dilutions were made using the same diluent, and 100 μ L of the final dilution was plated on GC agar with defined supplement and grown overnight at 37°C in a candle jar. The bacterial colonies were blotted onto circles of nitrocellulose membrane (BioRad Laboratories, Inc., Richmond, CA) by carefully placing the membrane on the surface of the agar for about 5 min. The membranes were then removed and immersed in blocking buffer containing 2% casein solution in PBS pH 7.4 and incubated with gentle shaking for 30 min. The membranes were then washed twice with PBS pH 7.4 and immersed in blocking buffer containing purified antibodies or rabbit serum at dilutions of 1:300. After overnight incubation at room temperature with gentle shaking, the papers were washed three times with PBS pH 7.4 and immersed in blocking buffer containing phosphatase-labeled goat anti-rabbit IgG (H+L) (Kirkegaard and Perry, Gaithersburg, MD) and gently shaken for 90 min. The membranes were then washed three times with PBS and once with 0.05 M Tris-HCl buffer pH 8.0. Bound antibody was detected by treatment of the papers with a solution containing naphthol phosphate as mixed salt (1 mg/mL) and fast red (2 mg/mL) (Sigma Chemical Co., St. Louis, MO) in 0.05 M Tris-HCl buffer pH 8.0. After incubation for 30 min with gentle shaking, the membranes were washed twice with water and air dried.

Test for stability of bactericidal antibodies in 3.0 M KCNS

The serum used for this test was a polyclonal human serum pool obtained from volunteers vaccinated with the tetravalent *N. meningitidis* (ACYW) polysaccharide vaccine. Serum (100 μ L) was diluted with 900 μ L of 3.4 M KCNS solution (final concentration, 3.0 M KCNS) and kept for 30 min at room temperature and then desalted on a PD-10 column with PBS pH 7.4 to remove thiocyanate. Another similarly treated sample was kept for 16 h at 5°C and then desalted on a PD-10 column with PBS pH 7.4. A third 100- μ L sample of serum was diluted with 900 μ L of 1.0 M NaCl and the mixture was kept for 16 h at 5°C and then desalted on the PD-10 column with PBS. A fourth 100- μ L sample of serum was diluted with 900 μ L of PBS pH 7.4 and kept for 16 h at 5°C and was then passed through the same PD-10 column for comparison. All desalted samples and the control sample were tested for bactericidal activity using *N. meningitidis* group C strain 60E.

Results

Isolation of anti-Lip antibodies

Anti-Lip antibodies were isolated from mouse monoclonal ascites, two convalescent patient sera marked X and Y, and the serum of rabbits that were immunized with purified Lip antigen. The anti-Lip antibody levels as determined by ELISA are shown in Table 1. It can be seen that the recovery of anti-Lip antibodies ranged from 60 to 80% of the total loaded onto the affinity column. The mouse monoclonal ascites fluid contained high levels of anti-Lip antibodies (28 400 units). The relatively low recovery of 60% may be accounted for by the fact that not all

TABLE 1. Anti-Lip antibody levels in whole sera and purified antibody preparations as determined by ELISA

Sera	ELISA antibody units in:		
	whole sera	anti-Lip	% recovery
Mouse ascites	28 400	17 000	60
Patient X	1 650	1 000	60
Patient Y	1 600	1 280	80
Rabbit (13 week)	2 000	1 600	80

NOTE: ELISA antibody units are the average of the reciprocal dilution multiplied by the absorbance at 410 nm taken at two serum dilutions with corresponding ODs between 0.3 and 1.2.

anti-Lip antibodies were eluted from the column with 3.0 M potassium thiocyanate, and by losses during processing of the samples. The mouse monoclonal antibodies were of the IgG isotype. The anti-Lip antibodies from convalescent patient sera X and Y were obtained in 60 and 80% yield, respectively. ELISA using phosphatase-labeled, affinity-purified, goat anti-human IgG, IgA, and IgM showed that anti-Lip antibodies from these sera belonged to all three immunoglobulin isotypes (data not shown).

Immunization of rabbits

Rabbits were immunized with purified Lip antigen (Bhattacharjee et al. 1988a) as described above. The anti-Lip antibody responses of two rabbits during the course of immunization are shown in Fig. 1. It can be seen that the primary immunization only resulted in about a 2-fold rise in antibody at 5 weeks, compared with the preimmunization level. A secondary antibody response was evident 2 weeks after the booster injection. The anti-Lip antibodies decreased somewhat between 7 and 9 weeks. The second booster injection was given at 11 weeks, and sera collected at 13 weeks showed a 4-fold rise in antibody compared with the level at 9 weeks.

Bactericidal activity

The bactericidal antibody titers of whole sera and affinity-purified anti-Lip antibodies against three strains of *N. meningitidis* group B are shown in Table 2. The convalescent patient serum X showed a bactericidal titer against all three strains of 1:80–1:160, but the isolated anti-Lip antibodies from this serum showed a very low bactericidal titer (1:4) against strain 8047 and no detectable bactericidal activity against the other two strains. The patient serum Y, which contained an equivalent level of anti-Lip antibody by ELISA, showed bactericidal titers against strains 44/76 and 8532 of 1:80 to 1:160, but was not bactericidal for strain 8047. The isolated anti-Lip antibodies from this serum failed to show bactericidal activity against any of the three strains tested. The immune rabbit serum (13 week) had a bactericidal titer of 1:16 against strain 44/76 but was not bactericidal for strain 8047. The isolated anti-Lip antibodies from this serum had no bactericidal activity against any of the three strains tested. The high-titer mouse monoclonal ascites (2-1-CA2) and a second mouse monoclonal anti-Lip ascites (12-1-CA2) failed to show bactericidal activity against any of the three strains tested (results not shown).

Colony blot assay

Colony blot assays were performed to verify the capacity of the purified anti-Lip antibodies to bind to whole bacterial cells. The results of assays using three strains of *N. meningitidis* group B and three antibody samples, namely (i) polyclonal anti-Lip

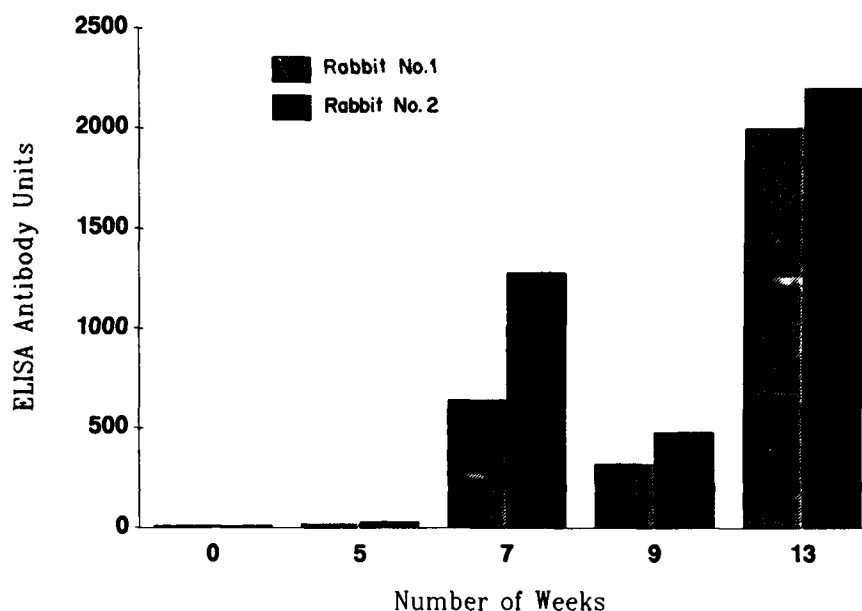


FIG. 1. Anti-Lip antibody response of rabbits during immunization with Lip antigen. Booster shots were given at 5 weeks and at 9 weeks after primary immunization.

TABLE 2. Bactericidal titers of affinity purified anti-Lip antibodies and of whole sera

Antibody source	Bactericidal titer ^a		
	Strain 44/76	Strain 8047	Strain 8532
Patient X			
Whole serum	80(64)	80(64)	160
Purified antibodies ^b	0	4	0
Patient Y			
Whole serum	80(80)	0	160(160)
Purified antibodies	0	0	0
Rabbit serum			
Prebleed	4	0	nt
7 week	8	4	nt
13 week	16	0	nt
Rabbit purified antibodies	0	0	0
Mouse monoclonal (2-1-CA2)	0	0	0

NOTE: The highest dilution of serum that gave greater than 50% killing of the test organism was taken as the titer.
nt, not tested.

^aNumbers in parenthesis show bactericidal titers of column wash fractions.

^bPurified, isolated anti-Lip antibodies.

rabbit serum, (ii) purified anti-Lip antibodies from rabbit serum, and (iii), preimmunization serum from the same rabbit are shown in Fig. 2. It can be seen that both anti-Lip rabbit serum and isolated anti-Lip antibodies from the rabbit serum showed binding to the bacterial colonies as revealed by black dots (actually red on color photograph) on the nitrocellulose membrane. The control preimmunization serum showed no binding to the colonies of the bacteria.

Stability of bactericidal antibodies in 3.0 M KCNS

The possibility that purified anti-Lip antibodies may have lost bactericidal function during purification was investigated by examining the stability of antibodies with known bactericidal activity in 3 M KCNS. The stability of bactericidal antibodies to group C polysaccharide in the polyclonal serum pool (ACYW)

in contact with 3.0 M KCNS solution is shown in Table 3. It can be seen that the serum treated with 3.0 M KCNS for 30 min at room temperature retained 88% of the original bactericidal activity for strain 60E. The serum treated with 3.0 M KCNS for 16 h at 5°C retained 62% of the bactericidal activity of the control serum. The serum treated with 0.9 M NaCl for 16 h at 5°C retained 100% of the original bactericidal activity.

Discussion

The Lip antigen that is common to the pathogenic *Neisseria* species (Cannon et al. 1984; Zollinger et al. 1985) has been found to be immunogenic in patients with meningococcal and gonococcal infections (Black et al. 1985). In view of the fact that at the present time there are no effective vaccines against meningococcal group B infections, and most experimental

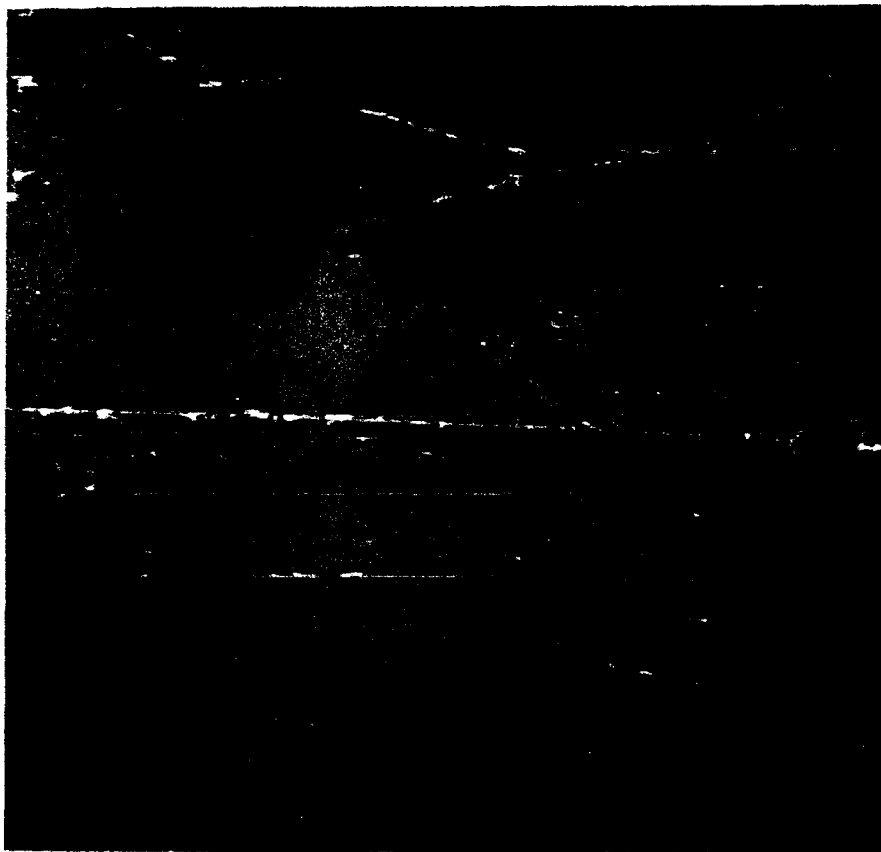


FIG. 2. Colony blot assay using three strains of *N. meningitidis* group B and rabbit sera containing anti-Lip antibodies. A, strain 44/76; B, strain 8047; C, strain 8529. Row 1, whole rabbit serum; row 2, isolated anti-Lip antibodies; row 3, preimmunization serum from the rabbit.

TABLE 3. Stability of serum bactericidal antibody in 3.0 M KCNS solution

Sample description	Bactericidal titer	% of original
ACYW serum in PBS, 16 h, 5°C	320	100
ACYW serum in 0.9 M NaCl, 16 h, 5°C	320	100
ACYW serum in 3.0 M KCNS, 30 min, room temperature	280	88
ACYW serum in 3.0 M KCNS, 16 h, 5°C	200	62

vaccines under evaluation are serotype specific, we wanted to explore the possibility of using Lip as a candidate vaccine. Since there is a strong correlation between the presence of serum bactericidal activity and human immunity to meningococci (Wong et al. 1977), the capacity to induce bactericidal antibodies is expected to be an important characteristic of an effective group B vaccine. We reported earlier (Bhattacharjee et al. 1988b) on the anti-Lip antibody titers of acute and convalescent sera from patients with meningococcal group B infection. It was found that a group of 10 convalescent sera had a geometric mean 15-fold rise in anti-Lip antibody as measured by ELISA using purified Lip as antigen. We selected two of these convalescent patient sera with high ELISA titers as shown in Table 1. The anti-Lip antibodies from these sera were isolated by affinity chromatography. The effectiveness of the Lip affinity column to retain anti-Lip antibodies was demonstrated by the absence of anti-Lip antibodies in the column wash

fractions. Since purified Lip (Bhattacharjee et al. 1988a) was used as the sensitizing antigen in the ELISA assay, comparison of ELISA antibody levels in the whole sera and in the isolated anti-Lip antibodies shows the actual recovery of these antibodies from the column. The dilution of the antibodies during the process of elution was taken into account in these calculations. As shown in Table 1, the recovery of anti-Lip antibodies from both mouse monoclonal ascites and the patient serum X were 60%. This relatively low recovery probably reflects the presence in this ascites and serum of some high-affinity antibodies which could not be eluted with 3.0 M KCNS. One of us has previously shown (Bhattacharjee and Glaudemans 1988) the presence of antibodies having high and low affinities for the same ligand in a polyclonal antibody pool. The anti-Lip antibodies from the patient serum Y and from the immune rabbit serum were isolated in 80% yield. The small losses in these cases can probably be accounted for by losses in working up the solutions. Tests for bactericidal antibodies showed (Table 2) that although both patient sera X and Y had relatively high bactericidal activity against the strains tested, the isolated anti-Lip antibodies from these sera had no bactericidal activity except serum X which had a titer of 1:4 against strain 8047. The numbers in parenthesis in Table 2 show the bactericidal titers of the column wash fractions. The results indicate that most of the bactericidal activity of these sera could be accounted for in the column wash fractions and therefore were not due to the anti-Lip antibodies that were retained by the affinity column. It may be mentioned here that the sera X and Y came from patients suffering from infection with meningococcal group B, serotype

15 strains, and were therefore homologous in serotype or subtype or both with two of the test strains, namely 44/76 and 8532. The high-titer mouse monoclonal ascites (2-1-CA2) and a second mouse monoclonal anti-Lip ascites failed to show bactericidal activity against any of the three strains tested. The bactericidal titer of the immune rabbit serum was low (1:16) against strain 44/76 and not detectable against strains 8047 and 8532, even though substantial anti-Lip antibody was present as judged by ELISA. Since the unfractionated sera had little bactericidal activity, it is not surprising that the column wash fractions in this case failed to show bactericidal activity. Hitchcock et al. (1987) reported that their Lip-specific McAb 10 gave approximately 0.5 log higher bactericidal activity than antibody-depleted normal human serum. It is noted that this was with a serum-sensitive strain of gonococci and that the monoclonal was used undiluted. The significance of this finding is not known at the present time.

The colony blot assay (Fig. 2) showed that purified anti-Lip antibodies were active in binding to whole cells or cell fragments of the three strains of meningococci that have been used for the bactericidal assay. This confirms the functional integrity of the antibodies in binding to native unprocessed antigen, but cannot resolve the issue of the extent of surface exposure of the Lip. Demonstration of bactericidal activity would constitute good evidence of surface exposure, but our failure to demonstrate such activity may indicate that such exposure is limited. The lack of bactericidal activity of the isolated anti-Lip antibodies was not due to the denaturation of the antibodies caused by the exposure to the chaotropic agent KCNS. As shown in Table 3, treatment of a polyclonal human serum pool with 3.0 M KCNS solution for 30 min at room temperature resulted only in minimal (12%) loss of bactericidal activity. This treatment is very similar to the condition used to isolate Lip-specific antibodies by affinity chromatography.

Our finding that anti-Lip antibodies isolated from convalescent patient sera and from immune rabbit serum lack significant bactericidal activity indicates that Lip may not be effective as a group B meningococcal vaccine. Whether these antibodies can provide protection through opsonization is not known at the present time.

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